Methods for the Isolation and Characterization of Constituents of Natural Products XIX. Use of a Celite–Sodium Borohydride Column for Reduction of Carbonyl Compounds at the Micro Level

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INTRODUCTION

This paper describes a column technique for carrying out sodium borohydride ($NaBH_4$) reductions of some simple carbonyl compounds at the microgram and micromole level. The procedure is rapid and easy involving only the application of a solution of the carbonyl compound to a column of Celite–$NaBH_4$ and collection of the effluent which contains the corresponding alcohol. For reductions on the microscale, at least, the technique is considered superior to the classical methods of performing $NaBH_4$ reactions (*1,3*) in that fewer steps are involved, the need for finding a suitable solvent system for the reactants is circumvented, and commercially pure or easily purified nonpolar solvents are employed.

MATERIALS AND METHODS

Celite 545 (Johns Manville Co., Baltimore, MD)[2] is dried overnight at 500–600°C. The dried Celite (4 g) and 1 g of $NaBH_4$ (Matheson Scientific, East Rutherford, NJ) are ground in a 4-inch mortar until homogeneous. The powder is stored in a tightly closed receptacle and kept in a desiccator over phosphorous pentoxide when not in use. It is active for at least 1 yr under these conditions.

n-Hexane (J. T. Baker, Phillipsburg, NJ) is rendered alcohol- and car-

[1] Research conducted while Dairy Products Laboratory was located in Washington, DC.

[2] Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

bonyl-free by passage over a chromic acid column (*4*) and then over a sulfuric acid column (*2*). It is finally distilled from KOH pellets. Carbon tetrachloride (Baker) and dichloromethane (Baker) were sufficiently free of carbonyl and alcoholic contaminants and were used without further treatment. All solvents were kept over calcium hydride.

Disposable Pasteur pipets (145 × 7 mm o.d.) were used for columns. They were cut just below the crimp to facilitate insertion of column materials and were plugged with a small wad of glass wool. For experiments at the microgram level, columns were made from melting point capillaries, 100 mm long, 1.5–2.0 mm o.d., which were cut approximately in half.

PROCEDURES AT THE MICROMOLE LEVEL

Procedure 1. Celite-$NaBH_4$ powder (300 mg) is transferred to a Pasteur pipet and tamped into a compact column 2.5–3.0 cm in length. Tamping should be firm enough to eliminate air pockets and to prevent the column from separating in the event that sufficient hydrogen is evolved to cause separation. On the other hand, the column should not be tamped excessively tight so that inordinately slow flow rates are obtained. A flow rate of 15–20 min/0.5 ml (after the column has been completely wetted with solvent) is adequate to reduce all but one of the carbonyl compounds studied to the corresponding alcohol in fair to excellent yield. Several practice runs are recommended to gain sufficient experience for preparing columns with the desired characteristics.

The carbonyl compound dissolved in hexane is pipetted onto the column and collection of the effluent is begun. When all of the solution has drained, the wall of the column is washed with a little hexane and when this has entered the bed, one column volume of dichloromethane[3] is added.

Procedure 2. The column is prepared as in procedure 1 except that 400 mg of the Celite–$NaBH_4$ powder is used. The hexane solution of the carbonyl compound (0.5 ml) is pipetted onto the dry column and when drained the wall is washed with a minimum of hexane. After these operations there should remain some unwetted portion of the column. After 10 min the alcohols are eluted as in procedure 1.

Quantitative aspects of the reduction were followed by assaying the effluent directly for the alcohol using the procedure of Schwartz (*4*). At least a 2.5 molar excess of pyruvic acid chloride 2,6-dinitrophenylhydrazone over the theoretical amount of alcohol was employed. The derivative was checked by thin-layer partition chromatography (*6*) against the expected authentic derivative.

[3] If the expected alcohol is known to be soluble in less polar solvents, e.g., hexane, benzene, CCl_4, etc., these may be used.

Procedure at the Microgram Level

Procedure 2 was modified so that it could be run on microliter volumes containing microgram or submicrogram amounts of carbonyls. A melting point capillary is dabbed into the Celite–$NaBH_4$ powder until a column of the mixture approximately 2.5 cm in length is retained. The powder is pressed into a compact column about 1.5 cm in length by using the ends of two paper clips or other suitable tampers. Up to 10 μl of a CCl_4 solution containing 0.5–5 μg of the carbonyl is injected onto the column and the wall washed down with 2 μl of CCl_4 from a clean syringe. After 10 min the column is eluted by injecting dichloromethane into the capillary. The first approximately 8 μl (about 8 mm) of solvent emerging contains the alcohol and is withdrawn with a hypodermic syringe. Light air or nitrogen pressure can be used to force the solution through the column.

Analysis of effluent for completeness of the reduction of carbonyls to the excepted alcohols was made by gas–liquid chromatography (glc). The conditions were: column, 4 ft × ⅛ inch silanized stainless-steel packed with 7.5% ethylene glycol adipate and 2% phosphoric acid on 90–100 mesh Anakrom ABS; instrument, Hewlett-Packard 5750; detector, flame; helium flow rate, 40 psi; injection port temperature, 230°C; detector temperature, 270°C; column temperature 55°–195°C programmed at 6°C/min. The range was set at 10 and the attenuation at ×4.

Retention time of authentic alcohols was used as evidence that the expected product had been obtained. When no authentic alcohol was available, a mass spectrum was used to identify the peak. The LKB-9000 gas chromatograph-mass spectrometer was utilized.

RESULTS AND DISCUSSION

The carbonyl compounds reduced at the micromole level using procedure 1 are listed in Table 1. Essentially identical results were obtained with Procedure 2 although not all of the compounds were investigated with Procedure 2.

The glc analysis of the alcohols produced in the reduction of the carbonyls at the microgram level indicated that all of the compounds examined were reduced to the expected alcohol with no or only traces of starting compound or extraneous peaks present. The following compounds were examined by this technique: acetophenone, benzophenone, 2,2-dimethyl-3-heptanone, furfural, 2-undecanone, 5-nonanone, menthone, 2-pentadecanone, 2-methylundecanal, 3,4-dimethoxybenzaldehyde, and cyclododecanone. The efficiency of the technique is illustrated in Fig. 1 which shows the reduction of tetradecanal, cinnamaldehyde, 2-undecanone, and benzophenone to the corresponding alcohols. The polyunsaturated fatty acid ester, methyl 5,8,11,14,17-eicosapentaenoate was included to see whether double bonds were

TABLE 1

CARBONYL COMPOUNDS REDUCED ON A CELITE–SODIUM BOROHYDRIDE COLUMN

Carbonyl compound	Amount over column (μmoles)	Alcohol found	Yield (%)
Aldehydes			
Benzaldehyde	2.6	Benzyl	100
Cinnamaldehyde	1.3	Cinnamyl	101
Crotonal	1.0	Crotonyl	67
2-Ethyl-2-hexenal	1.7	2-Ethyl-2-hexen-1-ol	90
Furfural	1.4	Furfuryl	63
2,4-Hexadienal	1.0	2,4-Hexadien-1-ol	104
5-Methylfurfural	0.5	5-Methylfurfuryl	65
2-Methylundecanal	1.0	2-Methylundecan-1-ol	104
Tetradecanal	1.3	Tetradecan-1-ol	100
Ketones			
Acetone	0.6	Isopropyl	80
Acetophenone	1.5	not identified	106
Benzophenone	0.8	Benzhydrol	95
Cholestan-3-one	1.3	Cholestan-3-ol	102
Δ5-Cholesten-3-one	0.8	Cholesterol	99
Cyclododecanone	1.0	Cyclododecanol	98[a]
Cyclohexanone	5.0	Cyclohexanol	101
Menthone	1.3	Menthol	86
3-Methyl-2-heptanone	0.9	3-Methyl-2-heptanol	99
Methyl-β-oxo-eicosanoate	0.8	not identified	107
Methyl-9-oxo-stearate	1.2	Methyl-9-hydroxystearate	93
5-Nonanone	2.2	5-Nonanol	100
2,7-Octanedione	0.5	2,7-Octanediol	96[b]

[a] Cyclododecanone gives a 40% yield of alcohol at a flow rate of 17 min/0.5 ml, a 98% yield in 37 min/0.5 ml.

[b] Determined by the procedure of Schwartz *et al.* (*5*).

reduced. The chromatogram indicated that double bonds are unaffected. Butyric acid was included in the mixture to demonstrate that organic acids are extracted but not reduced by the Celite–$NaBH_4$ column. Besides butyric acid the following acids were found to be completely extracted at the micromole level from a hexane or CCl_4 solution: stearic, tridecanoic, caproic, phenylacetic, benzoic, sorbic, 1-cyclohexene-1-carboxylic, and 3-cyclohexene-1-carboxylic.

Simple methyl and ethyl esters were not reduced at all under the prescribed conditions. However, some but not all Δ- and γ-lactones were partially or completely retained by the column, presumably after ring opening, but were not reduced. In addition, it was noted that some Δ-lactones were converted to γ-lactones by passage over the column. This interesting phenomenon is being further investigated.

In the micromole procedures, *n*-hexane as solvent gave better yields

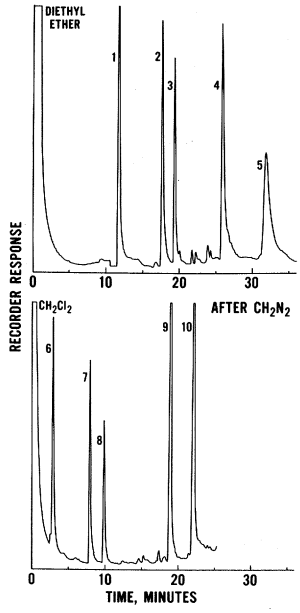

FIG. 1. Gas–liquid chromatograms of a mixture of aldehydes, ketones, an unsaturated ester, and an acid before (top) and after (bottom) contact with a micro column of Celite–$NaBH_4$.

of alcohol than did CCl_4, benzene or dichloromethane. In the microgram procedure, however, CCl_4 was substituted for hexane because of the tendency of the latter to tail and also to dirty the detector in glc.

Reductions with sodium borohyride are generally carried out in aqueous or alcoholic solution. With compounds that are poorly soluble in these solvents, dioxane–alcohol and ether–alcohol mixtures are sometimes used. Generally, regardless of the solvent used, the reduction products are isolated only after first decomposing the intermediate borates under acidic conditions. By using the column procedure, the product may be isolated directly, suggesting that no borate complex is formed under the prescribed conditions. The column procedure also offers an alternative to the use of oxygenated solvents.

SUMMARY

A Celite–sodium borohyride column has been used to reduce micromole amounts of a variety of simple carbonyl compounds to the corresponding alcohol in fair to excellent yields. The procedure is relatively rapid and easy involving only the application of a hexane solution of the carbonyl compound to the column and collection of the effluent containing the alcohol. A column constructed in a melting point capillary

suitable for the reduction of microgram amounts of carbonyls is also described.

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